

Influence of gender in pathogenesis of trichomoniasis in congenitally athymic (nude) mice

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SUMMARY Evaluation of abscesses appearing in male and female euthymic and athymic (nude) Balb/c mice after subcutaneous injection of *Trichomonas vaginalis* in the dorsal region showed that females were more susceptible than males. Female euthymic mice, however, were more susceptible than male athymic mice, and splenectomised athymic males were more susceptible than non-splenectomised athymic males. F₁ female athymic mice were the most susceptible, as their abscesses reached a peak size five days earlier than those of athymic homozygous females. F₁ male athymic mice, though slightly more susceptible than athymic homozygous males, did not develop abscesses that were similar in size. These results suggest that resistance or susceptibility to *T vaginalis* infection depends on the gender of the host and on thymus dependent cellular populations.

Trichomonas vaginalis is a cosmopolitan parasite that inhabits male and female genitourinary tracts.¹ In most men infection is symptomless and the clinical picture is uncertain,² whereas in women trichomoniasis produces pronounced vaginal inflammation, physical distress, and cytopathological tissue changes.^{3,4} Numerous workers have reported that the gender of the host affects the establishment, development, growth, or reproduction of a wide variety of protozoan and helminthic parasites.⁵⁻¹³

Experimental infection with *T vaginalis* in laboratory animals can be transmitted by different routes.¹ Our previous studies have shown the influence of gender and sex hormones on the uptake and development of *T vaginalis* infection in Balb/c mice.^{10,14} Athymic (nude) mice have been extensively used in the laboratory to evaluate the importance of T lymphocytes in the pathogenesis of metazoan and protozoan infections.^{15,18} The inability of athymic mice to mount effective cell mediated immune responses¹⁹⁻²¹ is thought to be due to their lack of a thymus.^{22,23} We therefore thought that it would be interesting to compare the responses of male and female athymic mice to infection with *T vaginalis* and evaluate the

importance of T cells in the pathogenesis of trichomoniasis.

Materials and methods

MICE

We used male and female six to seven week old Balb/c athymic mice and their white euthymic litter mates. In some experiments we used male and female F₁ Balb/c athymic mice, which had been obtained by mating homozygous athymic mice. The animals were maintained in sterile plastic boxes at 26°C and fed sterile pellet food (Piccioni, Brescia) and sterile water with antibiotics ad libitum.

PARASITES

Strain D of *T vaginalis* was isolated from a woman with acute vaginal infection, and was maintained axenically in modified Diamond's medium²⁴ supplemented with 10% heat inactivated fetal calf serum containing 100 IU/ml penicillin, 100 µg/ml streptomycin, and 20 IU/ml mycostatin. The stock preparation was then frozen at -80°C in 10% dimethylsulphoxide. Samples were thawed five days before use, and the challenge inocula were obtained after two passages in Diamond's medium. Virulence was evaluated before the beginning of these experiments by injecting progressive doses intraperitoneally in Balb/c mice and noting the speed of their deaths.

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SUBCUTANEOUS INFECTION

We injected 0.25 ml of a suspension in Hank's balanced salt solution containing 1×10^6 viable *T vaginalis* organisms subcutaneously in the dorsal region of 10 male and 10 female athymic mice and eight male and eight female white euthymic mice. Each animal was examined every other day from day 4 after injection in accordance with Honigberg's "subcutaneous mouse assay".²⁵

SPLENECTOMY

When splenectomy was required, we made a 5 mm long incision on the left upper flank. One or two stitches were enough to close the wound, which usually healed within one week.

STATISTICS

Using Student's *t* test, we compared the mean volumes of abscesses observed in different groups of mice. A difference of more than $p = 0.05$ was considered to be not significant.

Results

INFLUENCE OF GENDER ON *T VAGINALIS* INFECTION IN ATHYMIC MICE

Figure 1 shows that athymic females were more susceptible than athymic males. Their subcutaneous abscesses developed within four to six days and peaked

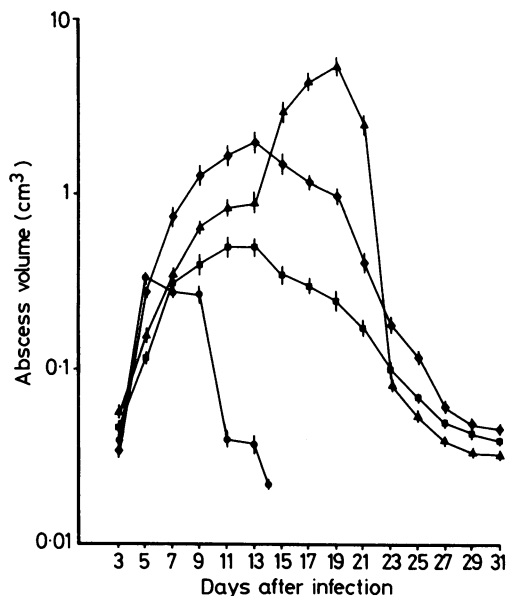


Figure 1 Mean (SD) abscess volume of eight euthymic male (●), eight euthymic female (◆), 10 athymic male (■), and 10 athymic female (▲) Balb/c mice after subcutaneous infection with *Trichomonas vaginalis*.

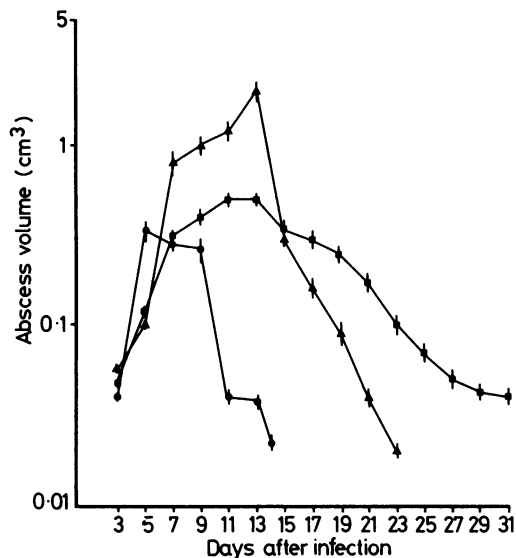


Figure 2 Mean (SD) abscess volume of eight euthymic male (●), eight athymic male (■), and 10 splenectomized athymic male (▲) Balb/c mice after subcutaneous infection with *Trichomonas vaginalis*.

after 17 to 20 days. By contrast, in athymic males the abscesses were always appreciably smaller than in athymic females, developed in four to six days, and peaked in 11 to 13 days.

Heterozygous males and females were more resistant than their homozygotes to subcutaneous infection, though euthymic females were more susceptible than euthymic males. Abscess volumes in male and female euthymic mice were appreciably less than in athymic mice.

INFLUENCE OF SPLENECTOMY OF ATHYMIC MALE MICE ON INFECTION WITH *T VAGINALIS*

Figure 2 shows that splenectomy did not influence the onset of abscesses, but after day 7 the volumes in splenectomized mice peaked to appreciably higher values. Moreover, these abscesses started to decrease more quickly (after 15 days) and disappeared in about 23 days.

T VAGINALIS INFECTION IN F_1 ATHYMIC MICE

To evaluate whether subsequent generations of male and female athymic mice maintained susceptibility that depended on their gender, we tested the F_1 animals of both sexes obtained by mating homozygous athymic mice.

F_1 female athymic mice were the most susceptible to *T vaginalis* infection (fig 3). Abscess sizes peaked in 13 to 15 days, five days earlier than observed in homozygous athymic females. F_1 male athymic mice,

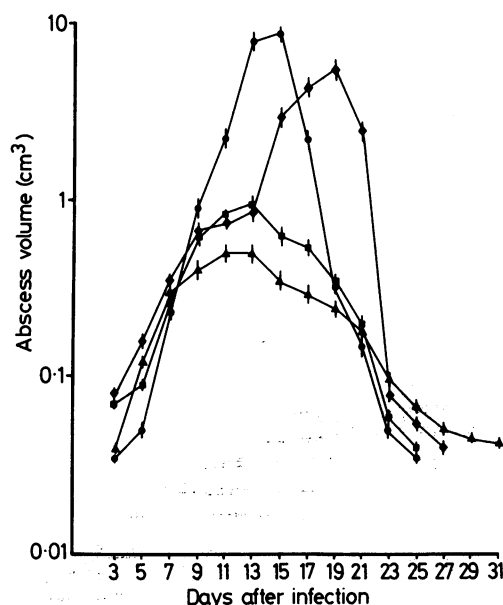


Figure 3 Mean (SD) abscess volume of 10 F_1 athymic female (●), 10 F_1 athymic male (■), 10 homozygous athymic female (◆), and 10 homozygous athymic male (▲) Balb/c mice after subcutaneous infection with *Trichomonas vaginalis*.

while slightly more susceptible than homozygous athymic males, did not develop appreciably larger abscesses.

Discussion

These results show clearly that susceptibility to *T. vaginalis* infection depends on gender in both euthymic and athymic mice (figure 1), that thymus dependent cell populations modulate both the onset and progress of infection, and that female hormones are important in increasing the possibility of a subcutaneous take.^{10,14}

Differences in the immune responses of males and females of the same species have often been described. The lymphocytes of males are more efficient effector cells in antibody dependent cellular cytotoxicity.²⁶ Females are more likely to develop autoimmune diseases.²⁷ Some workers have recently observed an influence of gender on the pathogenesis of *Trypanosoma rhodesiense*⁹ and *Leishmania major*⁸ infection in mice. Moreover, in the Middle East women are more prone than men to develop incurable recurrent leishmaniasis and display a poorer response to vaccination with attenuated *L. major* strains.²⁸

Splenectomy of athymic male mice did not

accelerate *T. vaginalis* takes, but did increase abscess size to values appreciably greater than in non-splenectomized athymic males (fig 2). Splenectomy, of course, deprived the athymic animals of an anatomical site rich in natural killer cells.²⁹

It is thus likely that both gender (and in particular its associated hormones) and pre-T cells co-operate to make males resistant to *T. vaginalis* infection. Furthermore, our previous work has shown that treatment of euthymic male mice with oestrogens enhances their sensitivity to subcutaneous trichomoniasis.¹⁴

F_1 athymic mice obtained by recrossing homozygotes maintained this gender linked sensitivity (fig 3). Pronounced differences were not noted between abscesses formed in F_1 males and females compared with their respective homozygotes. Here again, females were more susceptible than males. Abscesses did, however, develop more quickly in F_1 females than in their homozygotes. In males, too, there was a slight increase in abscess size, though the absence of oestrogens served to maintain a considerable degree of resistance.

In conclusion, athymic mice are more susceptible than euthymic mice to subcutaneous *T. vaginalis* infection. This emphasises the importance of the T cell mediated immune response in preventing trichomoniasis. As far as the greater susceptibility of females is concerned in both euthymic and athymic animals, treatment of female mice with diethylstilbestrol has recently been shown to decrease spleen cell production of interleukin-2, which results in enhanced susceptibility to *Listeria monocytogenes*.³⁰ Weakening the T cell response by oestrogens is therefore probably responsible for greater susceptibility in females.

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